

Monitoring *Tribolium castaneum* (Coleoptera: Tenebrionidae) in Pilot-Scale Warehouses Treated with Residual Applications of (S)-Hydroprene and Cyfluthrin

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ABSTRACT Pilot-scale warehouses, artificially infested with all life stages of *Tribolium castaneum* (Herbst), were used to evaluate the efficacy of two contact insecticides, (S)-hydroprene and cyfluthrin, and to determine the effect of insecticide treatments on insect captures in food- and pheromone-baited pitfall traps. Two application strategies were compared; insecticides were applied at the labeled rate either around the inside perimeter of the warehouse or in a band around the base of shelf units containing discrete food patches (10 g of wheat flour) infested with *T. castaneum*. Insect populations were assessed weekly for 6 wk by recording number of dead adults on the warehouse floor; number of larvae and adults captured in pitfall traps; and number of larvae, pupae, and adults recovered from food patch samples. There were significantly more dead adults in warehouses treated with cyfluthrin than with (S)-hydroprene or water (control treatment). However, food patch samples showed no detectable differences in quantity of larvae, pupae, or adults among any treatments. Pitfall traps detected fewer larvae starting the fourth week of the study in the warehouses treated with cyfluthrin around the shelf perimeter. Rate of larval capture in traps increased overall with increasing larval populations, but it was more pronounced in traps located closer to the food patches. Number of adults captured in pitfall traps reflected adult mortality in cyfluthrin-treated warehouses. Capture of larvae and adults was greater near the source of the infestation than elsewhere in the warehouse, suggesting that trapping data should be considered when precision targeting insecticide applications in the field.

KEY WORDS trapping, IPM, stored-product insects, contact insecticide, sampling

RESIDUAL CONTACT INSECTICIDES ARE commonly applied on a recurring basis in food-processing facilities, flour-mills, warehouses, and grocery stores for management of the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). These insecticides are generally applied to surfaces, spots, cracks, or crevices where the chemical residue persists at strength sufficient to kill at least one life stage of the target species encountering the treated surface. Based on mode of action, insecticides can be grouped broadly into several classes, including inorganics, organophosphates, insect growth regulators (IGRs), carbamates, and synthetic pyrethroids. Organophosphate and carbamate insecticides are undergoing a recertification process, and products may lose registrations because of the 1996 Food Quality Protection Act. Resistance to commonly used organophosphates is a serious problem (Subramanyam and Hagstrum 1996), and resistance

has been reported for *T. castaneum* recovered in U.S. grain-processing mills (Arthur and Zettler 1991, 1992; Zettler 1991). Reduced-risk insecticides such as IGRs (Oberlander et al. 1997, Mondal and Parween 2000, Oberlander and Silhacek 2000) may be viewed as possible replacement compounds, but limited data are available regarding the usage of these products despite a good record from an efficacy standpoint (Arthur 2003, Arthur and Hoernemann 2004).

Successful integrated pest management (IPM) for stored products (Hagstrum and Flinn 1996) requires a good monitoring system to obtain reliable information about insect populations (Burkholder 1990). Insect monitoring in food-processing, warehousing, and retail environments (Arbogast et al. 2000, 2002, 2005; Campbell et al. 2002, 2003; Roesli et al. 2003) is typically based on relative estimates of the insect populations because direct sampling of milled and packaged products is time-consuming and not economically feasible. The relationship between number of insects captured in pheromone traps and the actual population density is not always clear (Campbell et al. 2004); there is little published information on insect movement to pheromone-baited traps in mills or other

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field-type environments, and perimeter applications of contact insecticides could influence insect behavior near traps. Arbogast et al. (2003) showed *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae) adults were captured in pheromone-baited pitfall traps with greater frequency near the point of release in pilot-scale warehouses.

Studies in pilot-scale warehouses facilitate assessment of insecticide application strategies in an environment that permits insect movement and provides substantial refugia, conditions not possible in small laboratory studies. Conversely, experimental design with adequate controls and replication is difficult to achieve in commercial facilities. Additionally, measuring the impact of insecticides in field experiments can be difficult due to the inability to directly sample hidden insect populations and control insect immigration and emigration. Therefore, controlled experiments in pilot-scale warehouses were conducted to assess the impact of (S)-hydroprene (an IGR) and cyfluthrin (a synthetic pyrethroid) on populations of *T. castaneum*. The objectives of the study were to 1) evaluate the efficacy of the two insecticides and two application methods, and 2) investigate the effect of insecticide applications on insect captures in pheromone and food-baited pitfall traps.

Materials and Methods

Research was conducted in five climate-controlled pilot-scale warehouses with interior dimensions of 2.8 m in width by 5.9 m in length by 2 m in height. Warehouses were framed with wood and designed to prevent insect immigration and emigration via tight sealing of the inside plywood sheathing on the floor, walls, and ceiling; all permanent interior surfaces were sealed and coated with food-grade epoxy (see Toews et al. 2005 for details). Disposable floors were installed using the following procedures. Walls and floor of each warehouse were covered with a contiguous piece of 0.15-mm-thick polyethylene sheeting, and gray duct tape was used to seal the edges of the sheeting to the warehouse walls to prevent insect dispersal between the plastic sheeting and permanent interior walls. The entire floor was covered with 1.3-cm-thick gypsum panels (USG SHEETROCK brand, USG Interiors, Inc., Chicago, IL). Joints between gypsum panels were filled with three successive layers of joint compound (USG SHEETROCK brand) and sanded smooth, whereas floor-wall junctions between the gypsum panels and polyethylene sheeting were sealed with silicone sealant (Alex Plus, DAP Inc., Baltimore, MD). The gypsum panel-covered floor was coated with PVA drywall primer and sealer (part no. 73, Behr Process Corp., Santa Ana, CA) and top-coated with acrylic concrete floor sealer (part no. 900, Behr Process Corp.) to simulate a finished concrete floor in a commercial warehouse.

Environmental conditions were controlled to correspond to conditions found in commercial warehouses and processing plants. Temperature in each warehouse was maintained at $25.4 \pm 0.4^\circ\text{C}$ by using a

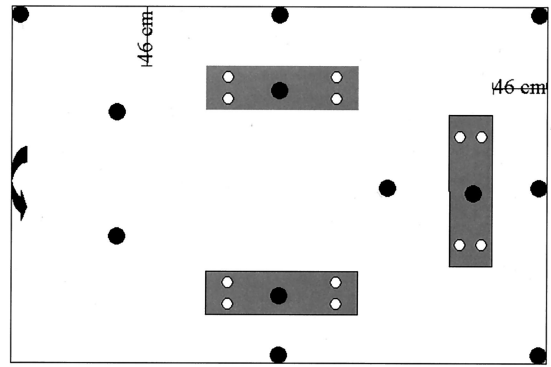


Fig. 1. Arrangement of shelves, traps, and food patches in pilot-scale warehouses during experiments. The arrow indicates the exterior door, gray rectangles indicate shelves, black circles indicate pitfall traps, and white circles indicate food patches (under shelves). Drawing is not to scale.

wall-mounted heating/cooling unit, whereas humidity fluctuated with ambient conditions ($29.3 \pm 0.8\%$). Temperature and relative humidity were recorded using a single HOBO data logger (Onset Computer Corporation, Bourne, MA) placed at ground level inside each warehouse. Two ceiling mounted 100-W incandescent light bulbs provided lighting: 42.0 ± 2.6 lx ($n = 20$), as measured at ground level by a digital light meter (model 401025, Extech Instruments, Waltham, MA). Photoperiod was 24:0 (L:D) h because most commercial warehouses operate day and night.

Refugia (shelves covering food patches) in each warehouse supported the insect populations. Each warehouse was equipped with three shelving units (Toews et al. 2005) that covered four food patches each (Fig. 1). Shelving units were positioned such that a sanitation buffer, a standard practice in commercial warehouses, was left between the interior warehouse walls and the shelving unit. Food patches (wheat flour, 10 g/patch) were placed on 50-cm filter paper disks underneath each shelving unit. Each food patch was infested with 17 eggs, 17 small larvae (second-third instars), 17 large larvae (seventh-eighth instars), 17 pupae, and 17 adults (1–2 wk old) of *T. castaneum*. During the study, 10 marked adults of mixed age and sex were released weekly to simulate insect immigration. Adults were marked by applying a small dot of waterborne acrylic enamel (General Paint & Manufacturing Co., Cary, IL) to the dorsal surface of the thorax with a microprobe (Fisher, Pittsburgh, PA).

Insecticides were applied in the pilot-scale warehouses 24 h after insect release. Separate hand-held applicators (model 406 CI, Solo, Newport News, VA), with Schrader valves for determining air pressure, were used to make the applications. Sprayers filled with finished solution were weighed on a balance before and immediately after each application to determine the actual quantity of solution applied. Insecticides evaluated during the experiment were cyfluthrin (Tempo 20 WP, Bayer Corporation, Kansas City, MO) and (S)-hydroprene (Gentrol IGR Concentrate,

Wellmark International, Bensenville, IL). Cyfluthrin was mixed at the high label concentration (0.1%) active ingredient (AI), whereas (S)-hydroprene was mixed at the only concentration on the label. Pesticide rate was calculated to match the label rate for general surface treatments, cyfluthrin at 3.79-liter finished solution per 92.9 m² and (S)-hydroprene at 3.79-liter finished solution per 139.4 m². Insecticides were mixed in distilled water on the day of application and thoroughly agitated before and during application. The applications were made at 137.9 kPa by using a hollow cone nozzle tip. Distilled water was applied in the warehouse designated as the control treatment. Two insecticide application strategies, perimeter and shelf, were evaluated. The perimeter application consisted of spraying the floor-wall junction around the interior perimeter of each warehouse, whereas the shelf application strategy consisted of spraying a band of insecticide on the floor around each of the three shelving units. The total quantity of finished insecticide solution applied on a per warehouse basis was 182.9 ± 32.7 ml for the (S)-hydroprene perimeter application, 140.4 ± 27.9 ml in the (S)-hydroprene shelf application, 262.4 ± 49.9 in the cyfluthrin perimeter application, and 167.7 ± 22.4 ml in the cyfluthrin shelf application. The shelf application required roughly 62% of the total quantity of material applied during perimeter application.

A three-step insect monitoring plan was initiated 24 h after insecticide application and continued weekly. Persons entering warehouses wore disposable polyethylene overboots (part no. 4BW-4573, Lab Safety Supply, Janesville, WI) that were changed between each warehouse to avoid residue contamination. First, dead adults were counted and collected from the floor of each warehouse. Second, relative estimates of insect populations were obtained using 12 pitfall traps (Dome trap, Trécé Inc., Adair, OK), baited with *T. castaneum* pheromone (CFB/RFB pheromone attractant, Trécé Inc.) and food oil, in each warehouse (Fig. 1). Trap position in each warehouse was characterized by presence in corners, along walls, in the middle, or under shelves. Third, to directly measure insect abundance in the food patches, an ≈ 6 -g sample of flour was collected from each warehouse (0.5 ± 0.1 g per flour patch and immediately pooled) by using a laboratory spatula. Pooled flour samples were taken to the laboratory, weighed, and sieved through a #60 U.S. standard testing sieve to determine the quantity of larvae, pupae, and adults present.

Experimental Design and Analyses. Number of dead adults, insects recovered in flour samples, and total number of marked individuals recovered in traps were analyzed as a split-plot arrangement of a randomized complete block design with repeated measures. The complete set of insecticide treatments was randomized to the five warehouses on three separate occasions, thereby providing three replications (or blocks) with each replication lasting 6 wk. After each replication, the temporary floors were removed and discarded, warehouses were thoroughly vacuumed and cleaned, and new temporary floors were installed

as described above. Individual warehouses were the main plot experimental unit for assessing effect of the insecticide applications. Week of study was the subplot variable; however, we treated week as a repeated measures effect and used the REPEATED statement of the PROC MIXED procedure (SAS Institute 1999) to model the variance-covariance relationship of the response variables among weeks. The chosen covariance structure to fit these repeated measurements across weeks was first-order autoregressive (Littell et al. 2002). Replication by treatment was specified as the subject for the repeated measures.

Numbers of adults and larvae captured in pitfall traps were analyzed as a split-split plot arrangement of a randomized complete block design with repeated measures. The main plot factor in these analyses was insecticide treatment, the subplot factor was trap position, and week of study comprised the sub-subplot treatment factor. The first-order autoregressive covariance structure also proved to be effective to model the intra-sample correlation for larvae captured in traps, whereas the unstructured covariance model was chosen for adults (Littell et al. 2002). Week of study was treated as a repeated measures factor with the subject specified as trap within replication by treatment. To normalize variances before analyses (Zar 1984), log transformation ($X' = \log_{10}(X + 1)$) was performed on counts of dead adults collected from floor and on counts of adults and larvae collected from pitfall traps. Means separation procedures, following the methods of Tukey (1949), were performed with the aid of a macro (Saxton 1998) designed specifically for use with PROC MIXED. Significant interactions were further analyzed using the slice option of the LSMEANS statement in PROC MIXED (SAS Institute 1999).

Results

Number of dead *T. castaneum* adults collected in each warehouse varied greatly among insecticide treatments (Fig. 2). Cyfluthrin treatments, regardless of application method, produced the highest adult mortality, approximately six-fold greater than (S)-hydroprene and 13-fold greater than the control treatment. There were no significant interactions between week of study and treatment ($F = 0.64$; $df = 20, 50$; $P = 0.86$), and week of study by itself did not have a significant effect on the number of dead adults ($F = 2.18$; $df = 5, 50$; $P = 0.07$).

Analyses of insect counts derived from direct sampling of food patches generally showed similar trends among each life stage. Most importantly, there were no significant differences in number of larvae, pupae, or adults (Fig. 2) among insecticide by application strategy treatments. There were no significant interactions between insecticide treatment and week of study for number of larvae ($F = 0.57$; $df = 20, 50$; $P = 0.92$), pupae ($F = 0.99$; $df = 20, 50$; $P = 0.49$), or adults ($F = 0.76$; $df = 20, 50$; $P = 0.74$). However, week of study contributed significant variation to each of these

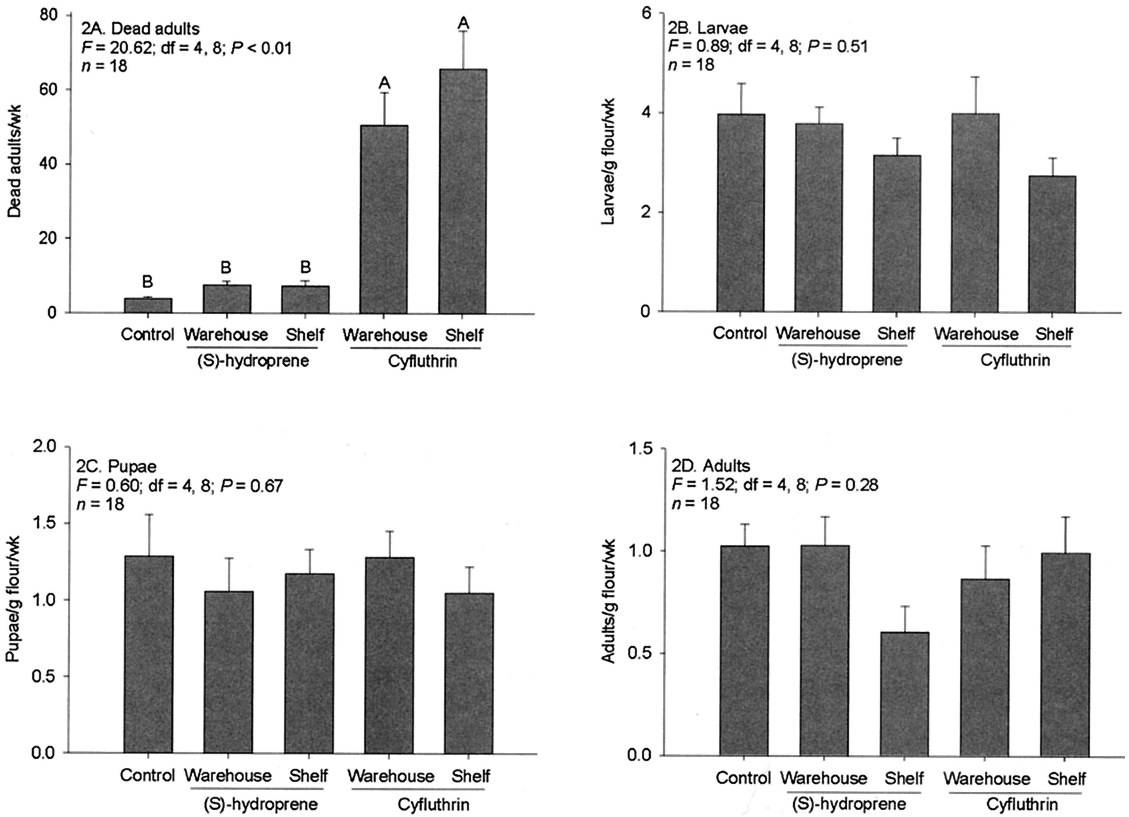


Fig. 2. Mean \pm SEM number of dead adults (A), larvae (B), pupae (C), or adults (D) of *T. castaneum* collected by week in pilot-scale warehouses by insecticide treatment. Dead adults were collected anywhere on the floor, whereas larvae, pupae, and adults were collected in direct food patch samples. Means with different letters above them indicate significant differences among treatments (Tukey test, $P < 0.05$).

response variables. Larvae increased over time ($F = 3.64$; $df = 4, 50$; $P < 0.01$) from 2.9 ± 0.5 larvae/g flour after the first week to a maximum of 4.5 ± 0.9 larvae/g flour. In contrast, pupae ($F = 13.90$; $df = 5, 50$; $P < 0.01$) and adults ($F = 2.67$; $df = 5, 50$; $P = 0.03$) decreased weekly throughout the 6-wk study.

Results of the pitfall trapping revealed that most *T. castaneum* larvae captured were large, late instars. Analyses indicated that there was not a three-way (treatment by trap position by week) interaction ($F = 0.70$; $df = 60, 919$; $P = 0.96$) for larval captures, but there were several two-way interactions. Week of study by insecticide treatment was significant because the number of larvae captured in warehouses receiving the cyfluthrin shelf application increased at a slower rate than the rest of the treatments (Fig. 3A). A second two-way interaction, week of study by trap position, was observed because the number of larvae captured over time increased faster for traps positioned under shelves compared with traps placed farther away from the food patches (Fig. 4). Traps placed in the corners captured very few larvae (0.6 ± 0.2) during the first week and showed little increase through the last week of the study (0.8 ± 0.2).

Similar to larvae, there was not a three-way interaction ($F = 0.71$; $df = 60, 919$; $P = 0.95$) in the analyses of adults captured in traps. However, we observed a significant insecticide treatment by week of study interaction (Fig. 3B). The number of adults captured decreased at a faster rate for the control and (S)-hydroprene treatments than the remaining cyfluthrin treatments. There were never any differences between the two cyfluthrin treatments regardless of week. Number of adults captured in traps also was affected by trap position (Fig. 5). More insects were captured under the shelves than in the remaining trap positions in each warehouse.

Very few marked individuals were captured in each warehouse, and there was no statistical difference among treatments in number of marked individuals recovered ($F = 1.50$; $df = 4, 8$; $P = 0.29$). There was no treatment by week interaction ($F = 1.14$; $df = 16, 40$; $P = 0.35$), but there was a strong effect attributed to week of study ($F = 3.56$; $df = 4, 40$; $P = 0.01$). Capture of marked adults ranged from was least after week 2 (0.3 ± 0.1), peaked after week 3 (1.3 ± 0.3), and then remained fairly constant through weeks 4 (0.9 ± 0.2), 5 (0.9 ± 0.3), and 6 (0.5 ± 0.2).

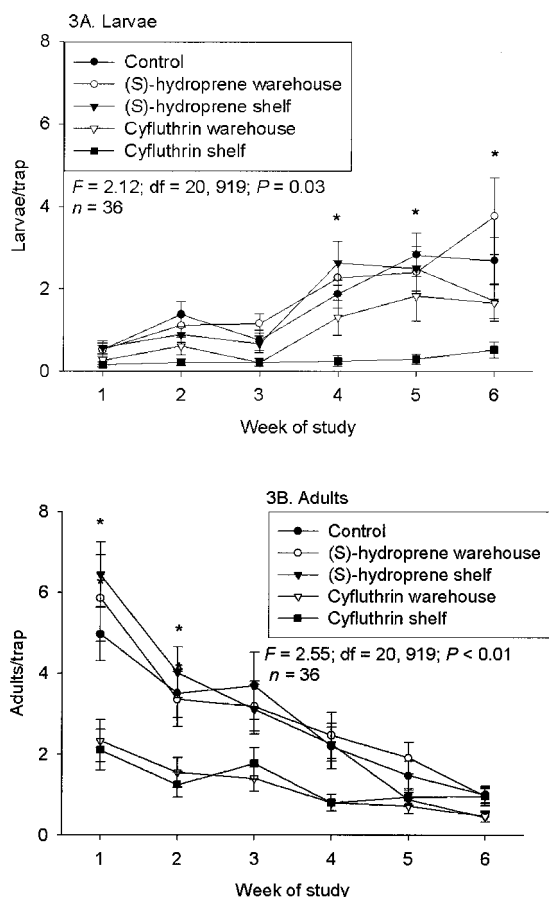


Fig. 3. Mean \pm SEM number of larvae (A) or adults (B) of *T. castaneum* captured in pitfall traps by insecticide treatment and week of study. Asterisks (*) indicate significant differences among insecticide treatments within individual week of study (Tukey test, $P < 0.05$).

Discussion

In this study, food patches containing *T. castaneum* were artificially manipulated to occur at prescribed locations within pilot-scale warehouses. Because foci of infestation were known, this allowed for assessment of pesticide efficacy through 1) direct sampling of the population from the food resource and 2) indirect monitoring of population changes and dispersal patterns based on captures in pitfall traps. In a commercial warehouse, the food source would be located most likely at an inaccessible location such as behind a hollow wall or ceiling or in an unknown crack or crevice. Because pest managers must rely on a relative measure of pest activity, such as pitfall trap captures, it is critical to see how the direct (food patch) and relative estimates differed.

Weekly collection of dead adults on the floor of each warehouse indicated that cyfluthrin applications were strongly impacting the adult life stage; however, no impact on populations directly sampled from the food patches was detected over the course of this

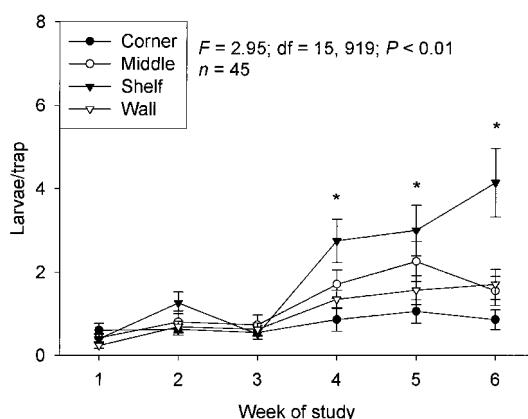


Fig. 4. Mean \pm SEM number of larvae of *T. castaneum* captured in pitfall traps by trap position and week of study. Asterisks (*) indicate significant differences among trap positions within individual week of study (Tukey test, $P < 0.05$).

study. The presence of dead insects may be interpreted by pest managers as sufficient evidence of successful control, but that initial observation may not reflect the true pest situation. Our results demonstrate the need to monitor insect populations beyond 1 or 2 wk after an intervention. Applications of (S)-hydroprene did not contribute significantly to adult mortality because IGRs target immature insects by preventing normal development to the adult stage.

Direct sampling of the food patches suggested that neither insecticide had a significant impact on the population developing within the food source. We have several hypotheses to explain these data. It is possible that the food patches were fully exploited even with the decreased adult numbers observed in the cyfluthrin-treated patches. Under this scenario, fewer eggs per female may have been deposited or more eggs were deposited but cannibalism and reduced fitness decreased progeny numbers in the control and (S)-hydroprene-treated warehouses. Alternatively, adults that survived in warehouses receiving

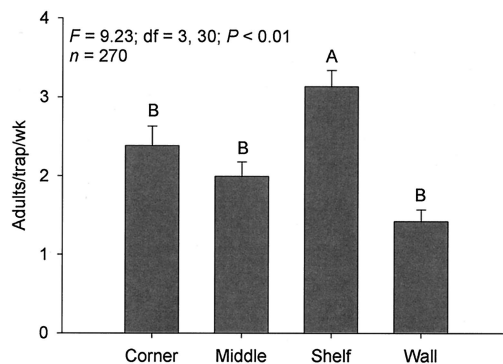


Fig. 5. Mean \pm SEM number of adults of *T. castaneum* captured in pitfall traps by trap position. Means with different letters above the columns indicate significant differences among trap positions (Tukey test, $P < 0.05$).

cyfluthrin applications may have deposited more eggs per female than adults in the other warehouses. In a controlled laboratory study, Campbell and Runnion (2003) demonstrated this phenomenon by placing the same number of beetles in variable patch sizes. Their data showed that *T. castaneum* females deposited proportionately more eggs in larger patches. We propose that insecticide applications in the field need to contact the food patches to significantly reduce the developing population. Hagstrum and Flinn (1992) postulated that a population of stored-product insects, after exposure to an intervention with 90% control, would regain its original population density after only 1 mo.

Week of study was a significant factor in the food patch analyses because the age structure of the population shifted from the proportion introduced initially (20% eggs, 40% larvae, 20% pupae, and 20% adults). Hagstrum et al. (1990) predicted the stable age structure to be ≈ 4 larvae per adult for another long-lived stored-product pest, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae). Their study predicted that larvae and pupae alone comprise 90% of the population. Perez-Mendoza et al. (2004) used direct sampling of bulk wheat to show the age structure for *R. dominica* to be 50.7% eggs and first instars, 42.2% second–fourth instars, 4.8% pupae, and 2.3% adults; and for *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae) to be 19.7% eggs, 70.7% larvae, 4.8% pupae, and 4.8% adults. Based on percentages reported for other stored-product beetles, it is predicted that adult *T. castaneum* make up $<5\%$ of the population, and even substantial adult mortality has little effect on overall population growth.

Indirect sampling indicated that some *T. castaneum* larvae could be captured in pitfall traps and the quantity captured could be correlated with the immature population increase through time observed in the direct samples. We hypothesize that some of the last-instar *T. castaneum* move away from the food patch in search of a pupation site, analogous to last instars of *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae). The significant differences in number of larvae captured among treatments in the fourth through sixth weeks of the study (Fig. 3A) indicated that the number of dispersing larvae was influenced by treatment regime; however, the number of dispersing larvae should not be confused with population of larvae in the food patches. Contact with (S)-hydrophrene prevents larvae from maturing to pupae; thus, more dispersing larvae may be present at a given time than would be expected under an untreated age structure. Loschiavo (1977) reported that *T. castaneum* larvae reared in hydrophrene-laced diets experienced lengthened larval developmental time and some failed to pupate. Cyfluthrin application probably decreased the number of dispersing larvae because of direct contact. It seems that very few *T. castaneum* larvae survived long enough to enter a trap after crossing the band of cyfluthrin in the shelf perimeter application. In general, we observed fewer larvae captured in traps placed away from the food patches, regardless of in-

secticide treatment. Pest managers should exploit immature captures by using them to pinpoint the infestation source.

Capture of dispersing adults in pitfall traps during the first 2 wk of the study reflected the cyfluthrin-induced adult mortality and the overall trend of decreasing adults per week observed for all treatment, including the control. Despite the decrease in adult captures, the number of larvae was increasing at the same time. In hindsight, it would have been worthwhile to follow the changing age structure of the population for a longer period or until it reached equilibrium. Similar to the findings of Arbogast et al. (2003), more insects were captured in traps closest to the source of the infestation. Toews et al. (2005) showed that correlations between the known adult insect population and captures in traps were stronger in warehouses with no food than those provisioned with food patches, whereas Campbell and Arbogast (2004) showed a correlation between *T. castaneum* pheromone trap captures and product infestation in flour mills. In summary, pitfall traps have been shown to be a reliable method for monitoring adult populations of *T. castaneum*.

The experiment was intended to cover the time needed for ≈ 1 generation of *T. castaneum* to develop, but we started with a balance of all life stages, so it was not necessary for all new eggs to complete development. Conducting the experiment for a longer period may have increased the efficacy of the (S)-hydrophrene treatments, but we included larvae and pupae in the starting insect population and expected to see some insecticidal effects attributed to the IGR. Because we did not directly treat the food patches, the most likely direct exposure opportunities were wandering larvae and adults. Additionally, adults may track residues into the food patch when returning for feeding or oviposition. Adult exposure to IGRs likely has no impact on future generations unless they carry residues to the food patch. Regardless, some pest management professionals are substituting IGRs with conventional insecticides without an understanding of the differences.

These data strongly suggest that application of contact insecticides requires much better precision targeting for measurable population suppression. Much of the existing literature on efficacy of contact insecticides to stored-product Coleoptera is based on no-choice exposure of individuals on surfaces (Burkholder and Dicke 1966; Williams et al. 1983; Arthur 1994, 1999a, b; Toews et al. 2003) or direct treatment of the media (Fang et al. 2002; Hou et al. 2004). Generally, laboratory studies show excellent results with all compounds. However, field populations may be able to avoid pesticide contact in time and space (Pinniger 1974; Barson 1991) or feed on untreated media after pesticide exposure, thereby increasing the probability of recovery (Arthur 2000a, b). Our data suggest that crack and crevice applications, unless applied to known refugia, are not an effective *T. castaneum* management strategy. Brenner et al. (1998) demonstrated an IPM tool, spatial analysis, which can

be used to precision target stored-product insect infestations. Additional work demonstrating the utility of spatial mapping (Arbogast et al. 2000, 2002, 2005; Campbell et al. 2002) or simple visualization of insect counts (Nansen et al. 2003) show how precision targeting can be used to enhance pest management. Use of these tools to target applications of contact insecticides will result in much greater efficacy than is possible with nontargeted applications.

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